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(57) Abstract

A recombinant human cytomegalovirus (HMCV) nucleic acid encoding a US11 protein or a US2 protein which lacks a functional first binding domain for class I MHC and which has a second binding domain for another target is described. Vaccines and methods for treating diseases with such constructs are also described. In addition, vaccines and methods for degrading class I MHC so as to treat autoimmune diseases or tissue graft rejections using recombinant nucleic acid constructs encoding HMCV US11 protein or US2 protein or functional portions thereof, are also described.

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CYTOMEGALOVIRUS NUCLEIC ACIDS ENCODING PROTEINS HAVING NORMAL OR ALTERED CLASS I MHC BINDING AND TREATMENT OF DISEASES

This application claims the benefit of U.S. Provisional Application No. 60/013,023 filed March 8, 1996.

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant No. 62832 awarded by National Institutes of Health.

Field of the Invention

The present invention relates to recombinant human cytomegalovirus nucleic acids having various binding domains for different targets, and methods for using such constructs for treating diseases.

Background of the Invention

T lymphocytes, T cells, have antigen-binding molecules on their cell surface called T-cell receptors that react with antigens on the surface of other cells. These T cells recognize other cells that display foreign antigens on their surfaces and kill the cells. For example, a T cell can target a virus-infected cell that displays fragments of a viral glycoprotein on its surface that is bound to a cellular protein called class I MHC (major histocompatibility complex). The peptide that becomes part of the complex of foreign peptide and class I MHC molecule, is derived from a protein that is first degraded and then released so as to become tightly bound to the MHC molecule. These peptides bound to the MHC molecules are displayed on the cell's surface, and the MHC molecules thus present the peptide to the T-cell receptor. Class I MHC binds peptides from intracellular proteins, e.g., peptides from viral proteins made in a virus-infected cell. The T cells, functioning in this way, thus are a principal immunologic defense mechanism against viruses.

The proteasome and transporter in antigen processing (TAP) genes are involved in the cytosolic proteolysis of the viral proteins and the delivery of the resulting peptides to the endoplasmic reticulum (ER), the exocytotic pathway, where they

can associate with newly synthesized chains of class I MHC molecules.

Certain viruses have evolved mechanisms which can manipulate the expression of the class I MHC gene products, so as to down-regulate such products, either by transcriptional or post-transcriptional mechanisms. It has been reported that in adenovirus infected cells, viral proteins are produced which retain the newly synthesized class I MHC molecules in the ER and thus prevent the MHC molecules from reaching the cell surface. It has also been reported that Herpes simplex viruses 1 and 2 produce a protein that blocks class I MHC cell surface expression by inactivating the TAP peptide transporter. This inactivation prevents access of the cytosolic peptides to the ER, and thus prevents stable assembly of class I MHC molecules and their expression at the cell surface. Human cytomegalovirus has also been reported to down-regulate expression of class I MHC molecules. (Beersma et al., J. Immunol. 151: 4455-4464 (1993); Jones et al., J. Virol. 69: 4830-4841 (1995)).

Summary of the Invention

According to the invention, a recombinant human cytomegalovirus nucleic acid encoding a US11 protein or a US2 protein which lacks a functional first binding domain for class I MHC and which has a second binding domain for a target, is provided. The target can be a viral protein, e.g., HIV GP20, an oncogenic element, e.g., a mutant form of Neu, an integrin, a selectin, a ligand, a receptor, a cytokine, a hormone, an antibody, an antigen, an enzyme, an enzyme substrate or a harmful agent.

Another aspect of the invention is a substantially pure protein encoded by the recombinant nucleic acid described above.

Another aspect of the invention is a recombinant vector comprising the recombinant nucleic acid described above.

Another aspect of the invention is a recombinant HCMV mutant comprising a genome which lacks a first nucleic acid sequence encoding a functional first binding domain for class I MHC, and which has a second nucleic acid sequence encoding a second binding domain for a target. The first nucleic acid sequence is gene US11, gene US2, or portions thereof.

Another aspect of the invention is a method for degrading a first protein. A mammal having cells which have a cytosol, is provided. A recombinant nucleic acid encoding a s cond protein, US11 or US2, which lacks a functional binding domain for class I MHC and which has a second binding domain for a target on the first protein, is provided. The recombinant nucleic acid is administered to the mammal under conditions which allow the second protein to interact with the first protein such that the first protein is degraded in the cytosol. Mammal is meant to include human and non-human mammals.

Another aspect of the invention is a method for treating a disease. A mammal having a disease, e.g., a viral infection, a bacterial infection, a malignancy, an autoimmune disease, or a transplant graft rejection, is provided. A recombinant nucleic acid encoding an HCMV US11 or US2 protein which lacks a functional first binding domain for class I MHC and which has a second binding domain for a target, e.g., HIV GP120 or class II MHC, is provided. The recombinant nucleic acid is administered, e.g., in a virus vector or in a non-infectious form, to the mammal in a therapeutically effective amount such that treatment of the disease occurs.

Another aspect of the invention is a vaccine composition for treating a disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding HCMV US11 or US2 which lacks a functional first binding domain for class I MHC and which has a second binding domain for a target, and a pharmaceutically acceptable carrier. In certain embodiments, the vaccine has an adjuvant and/or other therapeutic agents.

Another aspect of the invention is a pharmaceutical composition for treating a disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding HCMV US11 or US2 which lacks a functional first binding domain for class I MHC and which has a second binding domain for a target, and a pharmaceutically acceptable carrier.

Another aspect of the invention is a method for degrading class I MHC so as to treat an autoimmune disease. A mammal having an autoimmune disease, e.g., ankylosing spondylitis, is provided. The mammal has cells capable of synthesizing class I MHC. A recombinant nucleic acid encoding HCMV US11 protein, US2

protein, or functional portions thereof, is provid d. A therapeutically effective amount of the recombinant nucleic acid is administered to the mammal so as to degrade class I MHC such that treatment of the autoimmune disease occurs.

Another aspect of the invention is a vaccine composition for treating an autoimmune disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding HCMV US11 protein, US2 protein, or functional portions thereof, and a pharmaceutically acceptable carrier. In certain embodiments, the vaccine also has an adjuvant and/or other therapeutic agents.

Another aspect of the invention is a pharmaceutical composition for treating an autoimmune disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding HCMV US11 protein, US2 protein, or functional portions thereof, and a pharmaceutically acceptable carrier.

Another aspect of the invention is a method for degrading class I MHC so as to treat tissue graft rejection. A mammal having a tissue graft is provided. A recombinant nucleic acid encoding HCMV US11 protein, US2 protein, or functional portions thereof, is provided. A therapeutically effective amount of the recombinant nucleic acid is administered to the mammal so as to degrade class I MHC such that treatment of the tissue graft rejection occurs.

Another aspect of the invention is a method for degrading a first protein. A mammal having cells, the cells having a cytosol, is provided. A recombinant nucleic acid encoding a second protein, US11, US2 or functional portions thereof, which has a binding domain that is capable of interacting with the first protein, is provided. The recombinant nucleic acid is administered to the mammal under conditions which allow the second protein to interact with the first protein such that the first protein is degraded in the cytosol.

The above and other objects, features and advantages of the present invention will be better understood from the following specification when read in conjunction with the accompanying drawings.

Brief Description of the Drawings

FIG. 1 depicts an influenza hemagglutinin tag with flanking

sequences and restriction sites.

Detailed Description

This invention provides a recombinant human cytomegalovirus nucleic acid encoding a US11 protein or a US2 protein which lacks a functional first binding domain for class I MHC and which has a second binding domain for a target.

US11 and US2 are human cytomegalovirus (HCMV) genes. Chee et al., Curr. Top. Microbiol. Immunol. 154: 125-169 (1990); EMBL Gene Bank Database Accession No. X17403. US11 encodes an endoplasmic reticulum (ER) resident type-I transmembrane glycoprotein. A US11 or US2 protein is meant to include a protein with greater than about 60%, 70%, 80%, 90%, 95%, 98%, or 99% homology with the native US11 or US2 protein, respectively. US11 and US2 proteins each target newly synthesized class I MHC (major histocompatibility complex) heavy chains in the ER and redirect them to the cytosol. Following transfer to the cytosol, the class I MHC heavy chains are attacked by N-glycanase which removes the single N-linked glycan and converts the asparagine residues to which the glycan was attached to an aspartic acid. This change is diagnostic of N-glycanase activity, which is localized to the cytosol. Following removal of the N-linked glycan, the now cytosolic class I MHC molecule is destroyed by rapid proteolysis involving the cytosolic proteasome complex. Thus, the US11 and US2 proteins each result in down-regulating expression of class I MHC genes. See Examples 1-5.

By first binding domain for class I MHC is meant the region on the US11 or US2 protein which normally interacts with the class I MHC molecule. Interact is meant to include, e.g., bind, complex or associate. By lacking a functional first binding domain means that the normal US11 or US2 binding domain is altered so that it cannot properly interact with the class I MHC molecule. Such an alteration can result from, e.g., any type of mutation, e.g., a deletion, substitution, duplication, inversion, rearrangement or point mutation. Preferably, the first binding domain, or a functional portion thereof, is deleted.

By second binding domain is meant a binding domain which is

different from the first binding domain. In certain embodiments, the second binding domain results from altering the first binding domain, e.g., completely replacing the first binding domain with a second binding domain, or partially replacing the first binding domain so as to result in a second binding domain, or fusing the first binding domain or a portion thereof to a different amino acid sequence so as to result in a second binding domain, or deleting a portion of the first binding domain so as to result in a second binding domain, or altering in any other way the first binding domain so as to result in a second binding domain. In other embodiments, the second binding domain is in addition to an unaltered or altered first binding domain.

By whatever means the second binding domain is generated, it has a binding specificity for a different target than the first binding domain. Target is meant to include, e.g., a viral protein, e.g., HIV GP120, an oncogenic element, e.g., a mutant form of Neu, an integrin, a selectin, a ligand, a receptor, a cytokine, a hormone, an antibody, an antigen, an enzyme, an enzyme substrate, and a harmful agent. By harmful agent is meant a molecule that harms the cell. In certain preferred embodiments, the target is class II MHC. The second binding domain can be, e.g., an amino acid sequence capable of heterodimer formation. Second binding domains include, e.g., subunits of an adhesion glycoprotein or a functional portion thereof. By functional portion means a portion of the molecule that is able to bind to its target. Examples of second binding domains are a specific surface protein, e.g., CD8 or CD4 or functional portions thereof. By functional portion means a portion of the molecule that is able to bind to its target. In certain preferred embodiments, the second binding domain is CD4 or a functional portion thereof, that is capable of interacting with HIV GP120. In other embodiments, the second binding domain is a region of the Neu oncogene responsible for homodimer formation.

The invention also includes a substantially pure protein encoded by the recombinant nucleic acid described above.

The invention also includes a recombinant vector comprising the recombinant nucleic acid described above. By recombinant

vector is meant a vector having a nucleic acid sequence which is not normally present in the vector.

The invention also includes a recombinant HCMV mutant comprising a genome which lacks a first nucleic acid sequence encoding a functional first binding domain for class I MHC, and which has a second nucleic acid sequence encoding a second binding domain for a target. The first nucleic acid sequence is gene US11, gene US2, or portions thereof.

The invention also includes a method for degrading a first protein. A mammal having cells which have a cytosol, is provided. A recombinant nucleic acid encoding a second protein, US11 or US2, which lacks a functional binding domain for class I MHC and which has a second binding domain for a target on the first protein, is provided. The recombinant nucleic acid is administered to the mammal under conditions which allow the second protein to interact with the first protein such that the first protein is degraded in the cytosol. Mammal is meant to include human and non-human mammals.

The invention also includes a method for treating a disease. A mammal having a disease is provided. A recombinant nucleic acid encoding an HCMV US11 or US2 protein which lacks a functional first binding domain for class I MHC and which has a second binding domain for a target is provided. The recombinant nucleic acid is administered to the mammal in a therapeutically effective amount such that treatment of the disease occurs.

Disease is meant to include, e.g., a viral infection, a bacterial infection, a malignancy, an autoimmune disease, or a transplant graft rejection. Treating is meant to include, e.g., preventing, treating, reducing the symptoms of, or curing the disease. In certain embodiments, the method is used to treat HIV infection: the second binding domain is CD4 or a functional portion thereof, and the target is HIV GP120. In other embodiments, e.g., the second binding domain's target is class II MHC, and this method can thus be used to treat, e.g., an autoimmune disease, e.g., multiple sclerosis (HLA-DR2 linked disease), insulin dependent diabetes mellitus (HLADQw2, HLA-DQw8), rheumatoid arthritis (HLA-DR4), or juvenile rheumatoid arthritis (HLA-DP2, HLA-DR5/DR8), or it can be used, e.g., to treat graft rejection accompanying transplants, e.g., before or

after transplantation.

Autoimmunity is a phenomenon in which the host's immune response is turned against its own constituent parts, resulting in pathology. Many human autoimmune diseases are associated with certain alleles of class II MHC products. This association occurs because the structures recognized by T cells, the cells that cause autoimmunity, are complexes comprised of class II MHC molecules and peptides. The polymorphism of class II MHC genes in the human population ensures that genetically distinct individuals will display different MHC products, with unique binding specificities for peptide. Given the involvement of T cells in causing autoimmunity, it follows that certain class II MHC products will predispose for certain types of autoimmunity, while other allelic forms of class II molecules could be protective. It appears that the triggering of antigen-specific T cells, as brought about by infections with viral or bacterial pathogens, may unleash T cell clones that also react with the host's class II MHC products when complexed with peptides derived from the host's own gene products, resulting in autoimmunity. The different types of autoimmune diseases show strong associations with particular alleles at the HLA-DR and -DQ loci. According to this invention, alleviation of autoimmune symptoms can be brought about by eliminating the offending class II-peptide complexes from the surface of the antigen presenting cells in afflicted tissues or organs by redirecting the specificity of US11 or US2, through engineering of its binding domain. Destruction of class II MHC molecules is achieved prior to their complexation with offending self-peptides.

By therapeutically effective amount is meant that amount which is capable of at least partially preventing or reversing the symptoms of the disease. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the species of mammal, the mammal's size, the recombinant nucleic acid used, the type of delivery system used and the time of administration relative to the progression of the disease. A therapeutically effective amount can be determined by one of ordinary skill in the art

employing such factors and using no more than routine experimentation.

The recombinant nucleic acid can be administered to said mammal by any method which allows the recombinant nucleic acid to reach the appropriate cells. These methods include, e.g., injection, infusion, deposition, implantation, oral ingestion or topical administration. Preferably, administration is by injection. Injections can be, e.g., intramuscular, intravenous, intradermal, subcutaneous or intraperitoneal. The recombinant nucleic acid can be delivered, e.g., as part of a viral vector, e.g., avipox viruses, such as canary pox or fowl pox, recombinant vacciniavirus, replication-deficient adenovirus strains or poliovirus, or as a non-infectious form, e.g., naked DNA or liposome encapsulated DNA. Preferably, the virus will be administered by intramuscular injection in a dose range of about 10^3 to about 10^{10} infectious particles per injection, more preferably in a dose range of about 10^3 to abut 10^8 infectious particles per injection. Single or multiple doses can be administered over a given time period, depending upon the disease, as can be determined by one skilled in the art without undue experimentation.

The invention also includes a vaccine composition for treating a disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding HCMV US11 or US2 which lacks a functional first binding domain for class I MHC and which has a second binding domain for a target, and a pharmaceutically acceptable carrier. In certain embodiments, the vaccine also has an adjuvant. In other embodiments, the vaccine also has other therapeutic agents.

The invention also includes a pharmaceutical composition for treating a disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding HCMV US11 or US2 which lacks a functional first binding domain for class I MHC and which has a second binding domain for a target, and a pharmaceutically acceptable carrier.

The invention also includes a method for degrading class I MHC so as to treat an autoimmune disease. A mammal having an autoimmune disease is provided. The mammal has cells capable of synthesizing class I MHC. A recombinant nucleic acid encoding

HCMV US11 protein, US2 protein, or functional portions thereof, is provided. A therapeutically effective amount of recombinant nucleic acid is administered to the mammal so as to degrade class I MHC such that treatment of the autoimmune disease occurs. Any autoimmune disease that is associated with class I MHC can be treated by this method, e.g., ankylosing spondylityis (HLA-B27 linked disease).

The invention also includes a vaccine composition for treating an autoimmune disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding HCMV US11 protein, US2 protein, or functional portions thereof, and a pharmaceutically acceptable carrier. In certain embodiments, the vaccine also has an adjuvant. In other embodiments, the vaccine has other therapeutic agents.

The invention also includes a pharmaceutical composition for treating an autoimmune disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding HCMV US11 protein, US2 protein, or functional portions thereof, and a pharmaceutically acceptable carrier.

The invention also includes a method for degrading class I MHC so as to treat tissue graft rejection. A mammal having a tissue graft is provided. A recombinant nucleic acid encoding HCMV US11 protein, US2 protein, or functional portions thereof, is provided. A therapeutically effective amount of the recombinant nucleic acid is administered to the mammal so as to degrade class I MHC such that treatment of the tissue graft rejection occurs. Tissue graft rejection can occur e.g., as a result of tissue or organ transplants. Preferably, administration is accomplished by introducing the recombinant nucleic acid into the graft itself. Administration can be before or after transplantation. Preferably, administration is by liposome encapsulated nucleic acid.

The invention also includes vaccine compositions and pharmaceutical compositions for treating tissue graft rejections.

The invention also includes a method for degrading a first protein. A mammal having cells, the cells having a cytosol, is provided. A recombinant nucl ic acid encoding a second protein, US11, US2 or functional portions thereof, which has a binding

domain that is capable of interacting with the first protein, is provided. The recombinant nucleic acid is administered to the mammal under conditions which allow the second protein to interact with the first protein such that the first protein is degraded in the cytosol.

This invention is also meant to include similar constructs and methods for the other human cytomegalovirus Unique Short genes, US1 and US3-10, as those described above for US2 and US11.

EXAMPLES

Example 1: Class I MHC Molecules are Unstable in US11$^+$ Cells

The rapidity of the destruction of newly synthesized class I MHC molecules is apparent from an experiment in which US11$^+$ cells were pulse labeled for 60 seconds, followed by a chase up to 40 minutes. The amount of labeled class I heavy chains increased between the 0 and 1 minute chase points, which was attributed to the time required for equilibration of intracellular methionine and methionyl tRNA pools at these short pulses, and the time required for completion of the polypeptide chain. From 1 to 2 minutes, over half of the radioactivity in the class I heavy chains was lost and beyond 20 minutes of chase, no heavy chains remained. This rapid disappearance was not counteracted by a reduction of temperature: at 26°C or 16°C neither cessation of breakdown, nor the accumulation of breakdown intermediates was observed. Kinetics of labeling and destruction of heavy chains, not surprisingly, were slower at the reduced temperatures. It was concluded that breakdown of class I heavy chains takes place prior to their transport from the ER to the Golgi. Given the kinetics with which destruction occurred, the process is likely to be initiated immediately upon completion of the polypeptide chain. In cells that do not express US11, the class I heavy chains are stable over the time course observed here (Beersma et al., J. Immunol. 151: 4455-4464 (1993); Jones et al., J. Virol. 69: 4830-4841 (1995)).

Example 2: The Class I Breakdown Intermediate in US11 Cells is Resistant to Endoglycosidase H

Leucyl-leucyl-norleucinal (LLnL) treatment of US11$^+$ cells

inhibited a protease involved in class I MHC breakdown and led to accumulation of a 40 kDa breakdown intermediate. This finding indicated the possibility of a proteolytic cleavage removing the cytoplasmic tail. Removal of this fragment would produce an intermediate of the observed mobility, and proteolysis of the COOH-terminal cytoplasmic tail is a rather frequent occurrence for class I molecules. Surprisingly, antibodies raised against a synthetic peptide (SDSAQGSDVSLTA) (Seq. ID NO:1) derived from the cytoplasmic COOH terminus of human class I molecules still recognized the breakdown intermediate, indicating its COOH terminus was largely, if not entirely, intact. Other modifications of the class I heavy chain were considered, pulse-chase experiments in conjunction with digestion using the enzyme endoglycosidase H (endo H) were performed. Endo H cleaves high mannose-type oligosaccharides as commonly found on ER-resident N-linked glycoproteins. Class I complexes were recovered using the W6/32 antibody from control cells exposed to LLnL or BFA. The expected acquisition of partial endo H resistance was observed for untreated cells at 20 min of chase and for LLnL-treated cells; the reduction in total amount of W6/32 reactive complexes may again be attributable to reduction in peptide pools available for assembly (Rock et al., 1994). As expected, treatment with BFA at these relatively early chase points resulted in retention of full endo H sensitivity. For US11$^+$ cells, only minor amounts of assembled W6/32 reactive complexes were detected, almost all of which had disappeared by 20 min of chase. In US11$^+$ cells exposed to LLnL, the appearance of the 40 kDa intermediate was observed, which persisted for 20 min and was completely resistance to endo H. The mobility of the intermediate was indistinguishable from that seen for endo H-digested free heavy chains from control cells. It was concluded that the breakdown intermediate that transiently accumulates in LLnL-treated US11$^+$ cells contains little if any N-linked glycans.

Example 3: N-Linked Glycans Are Removed from Class I Molecules in US11$^+$ Cells by an N-Glycanase Type Activity

A pulse-chase experiment performed on US11$^+$ cells in the

absence or presence of LLnL established the precursor product relationship of the 43 and 40 kDa forms. Even though LLnL slows down proteolysis, it cannot prevent it: by 40 min of chase, most of the heavy chains synthesized in the 45 s pulse had disappeared.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) failed to distinguish between class I heavy chains deglycosylated using endo H and class I heavy chains isolated from tunicamycin (TM)-treated cells; these differ by the presence of a single GlcNAc residue. The possibility of an N-glycanase-type reaction was considered, in which the N-glycosidic bond is hydrolyzed, and the Asn sidechain is converted to an Asp residue (reviewed by Tarentino and Plummer, Meth. Enzymol. 230: 44-57 (1994)). Such conversion should result in a change in isoelectric point of the class I heavy chains.

When the samples were analyzed by one dimensional isoelectric focusing (IEF), multiple bands were observed in the anti-heavy chain precipitates. This multiplicity of bands was due to the simultaneous presence of the products of HLA-A, -B, and -C loci, each with a unique isoelectric point (Neefjes et al., Immunogenetics 23: 164-171 (1986)). Most of the class I heavy chains have isoelectric points well above pKa of sialic acids or protein-bound phosphate moieties (Eichholtz et al., J. Biol Chem. 267: 2490-2495 (1992)). For each of the isoelectric species present at the beginning of the chase, the breakdown intermediate seen in LLnL-treated cells at later time points migrated at a more acidic isoelectric point. Based on previous observations (Eichholtz et al., J. Biol. Chem. 267: 2490-2495 (1992)), this change in isoelectric point was of a magnitude comparable to that seen for addition of a single sialic acid residue and thus most likely corresponded to the conversion of the amide (Asn) to the acid (Asp). For comparison, class I molecules isolated from TM-treated cells were included. In TM-treated cells glycosylation is suppressed, and the acceptor Asn residues persist as such without affecting isoelectric point. The presence of the single remaining GlcNAc residue as produced by digestion with endo H should not affect the isoelectric point of the class I heavy chains either, and ind ed yielded digestion products indistinguishable in focusing pattern from class I

heavy chains isolated from TM-treated cells. In vitro treatment of core-glycosylated class I h avy chains with peptide N-glycanase yielded class I heavy chains with isoelectric points identical to the breakdown intermediates seen in LLnL-treated US11$^+$ cells, and the breakdown intermediates in US11$^+$ cells were refractory to digestion with N-glycanase. Combined, these data indicate that the breakdown intermediate has lost all N-linked oligosaccharides in an N-glycanase catalyzed reaction, the polypeptide backbone remaining intact.

Example 4: The Proteasome Inhibitors Carboxybenzyl-Leucyl-Leucyl-Leucinal and Lactacystin Produce a Class I Breakdown Intermediate Indistinguishable From That Seen in LLnL-Treated Cells

Peptide aldehydes such as LLnL have been widely used as inhibitors of proteases (Sherwood et al., Proc. Natl. Acad. Sci., USA 90: 3353-3357 (1993); Rock et al., Cell 78: 761-771 (1994)). The precise target of inhibition is often difficult to assess, and the rank order of inhibitory potency on purified proteases derived from different sources, such as lysosomal or proteasomal, has been used as a criterion for identification of their intracellular targets (Rock et al., Cell 78: 761-771 (1994)). For example, whereas LLnL has been described as a calpain I inhibitor, it is known to inhibit other proteases, including lysosomal proteases and the proteasome (Rock et al., Cell 78: 761-771 (1994)). The compound carboxybenzyl-leucyl-leucyl-leucinal (Cbz-LLL) was described as a very potent inhibitor of proteasomes (Rock et al., Cell 78: 761-771 (1994)). This compound was synthesized using solution chemistry and was found to produce a class I breakdown intermediate indistinguishable from that produced in the presence of LLnL. The antibiotic lactacystin (Fenteany et al., Science 286: 726-731 (1995)) is perhaps the most specific inhibitor of proteasomes discovered yet: radioactive lactacystin labels only the β-type subunit of proteasomes, and the compound is strongly inhibitory on proteasomal proteolysis (Fenteany et al., 1995). Lactacystin, like LLnL and Cbz-LLL, produced the 40 kDa breakdown intermediate. It was concluded that the specificity profile of inhibition indicated the involvement of proteasomes in the destruction of class I heavy chains in US11$^+$ cells.

Example 5: Subc llular Fractionation Shows Dislocation of the Class I Heavy Chain from the Microsomal Fraction to the Cytosol

Subcellular fractionation experiments were performed on control cells and US11$^+$ cells labeled in the presence of LLnL. For both control and US11$^+$ cells, the 1000 g pellet contained substantial amounts of class I heavy chains, β_2m, calnexin, and transferrin receptor (TfR). This pellet may contain unbroken cells, and larger cellular debris that may also trap soluble proteins. Therefore, the distribution of the proteins of interest over the 10,000 and 100,000 g pellets and the 100,000 g supernatant were considered to be the more significant parameters. In US11$^+$ cells, both intact heavy chains and some of the breakdown intermediates were present. Whereas in control cells, almost all class I heavy chains were recovered in the 1000 and 10,000 g pellets and no class I heavy chains were detectable in the 100,000 g supernate. The breakdown intermediate seen in LLnL-treated US11$^+$ cells was largely recovered from the 100,000 g supernate. Therefore, US11 mediated the transfer of the class I heavy chain from the ER environment to the cytosolic compartment. In the same subcellular fractions, the light chain β_2m that was not associated with class I heavy chains (no coprecipitating heavy chains were observed) sedimented predominantly with the microsomal fractions, and its distribution was not noticeably different for control cells and US11$^+$ cells. The intracellular compartments that contain β_2m must therefore remain largely intact in the course of homogenization. This finding also argues against breakage of intracellular organelles as the source of the soluble class I breakdown intermediate in US11$^+$ cells, and supports its cytosolic localization. Note that recovery of the class I breakdown intermediate using anti-heavy chain serum did not require the inclusion of detergent.

Example 6: Identification of the Binding Domain for Class I MHC on the US11 Protein

By introducing an epitope tag (influenza HA tag) in different segments of the US11 gene product, to be accomplished using PCR-based standard oligonucl otide directed mutagenesis,

the entire US11 sequence is scanned, and mutants are identified that carry the epitope tag, but have lost the ability to engage in dislocation. In addition, an epitope tagged version of US11 is prepared, where the epitope tag (flue HA) is carried at the extreme C-terminus. This arrangement allows production of a series of progressive truncations from the N-terminus inward. Each of these truncation variants is examined for the ability to mediate dislocation of class I molecules. The combination of epitope tag scanning, and truncation analysis identifies the region of US11 responsible for interaction with class I molecules. This identification is further narrowed down by site directed mutagenesis. The procedures by which this is accomplished are as follows:

The epitope tag used is influenza hemagglutinin tag, with amino acid sequence as shown in Figure 1. This sequence is flanked by a number of restriction sites (see Figure 1) that allow facile insertion into a suitable cloning vector. Primers complementary and in frame with the tag, and extended at the 5' end with a sequence complementary to the 3' end of the US11 coding sequence are used to allow amplification by PCR of a sequence that translates into a tagged US11 open reading frame, where the tag is at the COOH-terminus of US11. This primer has the sequence 5' CCATCCCTATGCGTAGTCTGGTACGTCGTATGGGTAGGCCATTCCGGG CCCCCACTGGTCCGAAAA 3' (Seq. ID NO:2) and is used in conjunction with a primer corresponding to the 5' end of US11, of the sequence 5' ATGAACCTTGTAATGCTT 3' (Seq. ID NO:3). This pair of primers, used in a polymerase chain reaction, yields an amplified product that contains the US11 reading frame fused in frame to the HA epitope tag. This amplified fragment is recloned into a suitable expression vector, pDCMV8, to allow expression of the tagged construct in HeLa cells following transfection. Progressive deletions of US11 are made by cloning the tagged construct into a cloning vector that can be replicated as single strand, in conjunction with PCR on the single strand using primers that contain the sequence 5'ATGAACCTTGTAATGCTTATTCTAGCCCTCTGGGCCCCGGTCGCGGGTAGT 3' (primer US11 5') (Seq. ID NO:4) immediately followed by a 15 base sequence, in frame, starting at the position where the new N-terminus is designed to be. As an example, to delete the first

20 residues of the US11 sequence, the US11 5' primer is extended with the sequence 5' GTGGAGACGGAGCCG 3' (Seq. ID NO:5). This primer is now used on the intact, tagged US11 sequence, in conjunction with a second primer of sequence 5' GGAATTCCTATGCGTA 3' (Seq. ID NO:6) in a PCR reaction. Amongst the amplified product are sequences with the desired deletion, to be identified by sequence analysis. These deleted constructs encode a product that contains the cleavable US11 signal sequence, but with progressive deletions from the NH2 terminus, and having the HA tag at the COOH terminus. Upon transfection of these deletion mutants, their expression is verified in a metabolic labeling experiment in which the US11 mutant product is identified by immunoprecipitation with the anti-HA epitope tag antibody, the effects of the mutant US11 products on dislocation of class I molecules are assessed as follows:

The effects of the mutant US11 products on dislocation of class I molecules is assessed by metabolic labeling of cells transfected with the mutant US11 products, followed by the immunoprecipitation of the target molecules, e.g., the MHC class I molecules. Separation of the immunoprecipitates on SDS-polyacrylamide gel or isoelectric focusing gel and visualization by fluoragraphy allows quantitation of the US11-induced degradation of the MHC molecules. Alternatively, steady state levels of the target molecules are determined by immunoblotting of the immunoprecipitated and SDS-PAGE-separate target molecules.

The fate of the target molecules is followed in time by pulse-chase analysis: after very short (45-60 seconds) metabolic labeling of the cells transfected with the mutant US11 molecules, the cells are chased in the presence of excess non-radioactive amino acids. Thus, the half-life of the target molecules in the presence of the US11 mutants is established. Inclusion in the media of protease inhibitors such as Carboxybenzyl-Leucyl-Leucyl-Leucinal (Cbz-L3) or lactacystin, both known to inhibit proteasomal degradation, allows the identification of breakdown intermediates. A polyclonal antiserum recognizing multiple epitopes occurring throughout the target molecule are used to detect such breakdown intermediates.

Dislocation of the target molecules to the cytosol by the

mutant US11 molecules is monitored by subcellular fractionation of metabolically labeled transfectants, followed by immunoprecipitation of the target molecules from the subcellular fractions obtained. Again, inclusion of protease inhibitors allows monitoring of both the intact target molecules and degradation intermediates.

This strategy allows the minimal size of US11 that is required for its dislocation activity on class I molecules. By performing transient transfection assays in Cos cells (See "Lipofectine" system, available from Gibco BRL Life Technology), each of these mutant constructs is overexpressed. In Cos cells that overexpress wild type US11, a sizable fraction of US11 escapes from the ER and reaches the Golgi apparatus and/or cell surface, where dislocation of class I molecules is no longer possible. For this reason, overexpression of wild type US11 in Cos cells allows the detection by immunoprecipitation of a physical complex between US11 and class I heavy chains. Progressive deletion of sequences from the N-terminus of US11 abrogates interactions with class I molecules, without necessarily affecting the dislocating activity of the remainder of the US11 product.

Example 7: Altering the Binding Domain for Class I MHC On the US11 Protein

The following two strategies are followed to engineer the binding site of US11 such that it will acquire specificity for a new protein of interest. In the following it is assumed that the transmembrane segment and cytoplasmic domain, in addition to a minimal region of the lumenal domain of US11 suffice to mediate the dislocation reaction, whereas the remainder of the lumenal domain of US11 determines the preference in terms of the protein that is targeted for dislocation.

(a) A US11 Variant That Binds to GP120

The region on CD4 that is involved in binding to HIV GP120 has been delineated by mutagenesis. It is located on domain 1 of CD4, and comprises residues 1-71. A segment of CD4 containing the corresponding region of the intact CD4 cDNA is fused to US11, as follows:

Starting from the CD4 sequence (see Hodge et al., Human

Immunology 30(2): 99-104 (1991)), amino acid residues 1-80 of the CD4 molecule are introduced into US11 by synthesis of the primers 5' CAAGCCCAGAGCCCT 3' (primer 1) (Seq. ID NO:7) and 5'GTTTTTCCAGTGGAAT 3' (primer 2) (Seq. ID NO:8) corresponding to residues 1-5 and 77-81 respectively of CD4. These primers are extended at the respective 5' ends with sequences 20 base pairs in length, complementary to and in frame with the sequence of US11 abutting the designated point of insertion. These primers are used to amplify by polymerase chain reaction the 1-81 segment of CD4, as well as the short regions of US11 flanking the CD4 sequence, as produced by the polymerase chain reaction. By cloning the US11 cDNA into an M13-derived vector that can be propagated in single stranded form, annealing of the extended CD4 fragment with the single stranded US11 target DNA and synthesis of the complementary strand with polymerase I, mutants that have the appropriate insertions at predesigned locations are obtained. In addition, domains 1 and 2 (residues 1-160) of CD4 are fused into US11, as outlined for the fusion of domain 1 (see above). The locations of insertions of the CD4 domain 1 and the combined domains 1 and 2 are designated in accordance with the identification of the binding site outlined above, such that the class I binding site identified in US11 is replaced with domain 1, and domain 1 and 2 of CD4 respectively.

(b) A US11 Variant That Binds to Class II MHC Molecules

To target the class II β chain for destruction in a US11-facilitated manner, the immunoglobulin-like domain of the class II α chain is fused to US11. This fusion product displays affinity for the class II β chains, and in doing so, targets them for destruction by dislocation to the cytosol. This construct will be generated as follows: 5'GAGCCCAACGTCCTC 3' (pos. 123-128 of DRα) (Seq. ID NO:9) and 5'AGGCTCATCCAAGCCCCA 3' (192-198 of DRα) (Seq. ID NO:10).

Those skilled in the art will be able to ascertain, using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Ploegh, Hidde L.
Wiertz, Emmanuel J.H.J.

(ii) TITLE OF INVENTION: RECOMBINANT HUMAN CYTOMEGALOVIRUS NUCLEIC ACIDS ENCODING UNIQUE SHORT PROTEINS HAVING NORMAL OR ALTERED CLASS I MHC BINDING DOMAINS AND THEIR USE IN TREATING DISEASES

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Nutter, McClennen & Fish, LLP
(B) STREET: One International Place
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02110-2699

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC Compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Word Perfect 6.0 DOS

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Not available
(B) FILING DATE: 06-MA-1997
(C) CLASSIFICATION: Not available

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Greer, Helen
(B) REGISTRATION NUMBER: 36,816
(C) REFERENCE/DOCKET NUMBER: 23160-36

(ix) TELECOMMUNICATION INFORMATION
(A) TELEPHONE: 617-439-2781
(B) TELEFAX: 617-973-9748

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ser Asp Ser Ala Gln Gly Ser Asp Val Ser Leu Thr Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCATCCCTAT GCGTAGTCTG GTACGTCGTA TGGGTAGGCC ATTCCGGGCC CCCACTGGTC 60

CGAAAA 66

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGAACCTTG TAATGCTT 18

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATGAACCTTG TAATGCTTAT TCTAGCCCTC TGGGCCCCGG TCGCGGGTAG T 51

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGGAGACGG AGCCG 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAATTCCTA TGCGTA 16

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAAGCCCAGA GCCCT 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTTTCCAG TGGAAT 16

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAGCCCAACG TCCTC 15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGGCTCATCC AAGCCCCA 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGCCC GGA ATG GCC TAC CCA TAC GAC GTA CCA GAC TAC GCA 42
Gly Met Ala Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
1 5 10

TAGGGATCC 51

CLAIMS

1. A recombinant human cytomegalovirus nucleic acid encoding a protein selected from the group consisting of US11 protein and US2 protein, said protein lacking a functional first binding domain for class I MHC and said protein having a second binding domain for a target.

2. The nucleic acid of claim 1 wherein said first binding domain, or a functional portion thereof, is deleted.

3. The nucleic acid of claim 1 wherein said second binding domain replaces said first binding domain in said protein.

4. The nucleic acid of claim 1 wherein said target is selected from the group consisting of a viral protein, an oncogenic element, an integrin, a selectin, a ligand, a receptor, a cytokine, a hormone, an antibody, an antigen, an enzyme, an enzyme substrate, and a harmful agent.

5. The nucleic acid of claim 4 wherein said viral protein is human immunodeficiency virus GP120.

6. The nucleic acid of claim 4 wherein said oncogenic element is a mutant form of Neu.

7. The nucleic acid of claim 1 wherein said target is class II MHC.

8. The nucleic acid of claim 1 wherein said second binding domain is an amino acid sequence capable of heterodimer formation.

9. The nucleic acid of claim 1 wherein said second binding domain is CD4 or a functional portion thereof.

10. The nucleic acid of claim 9 wherein said CD4 or said functional portion thereof is capable of interacting with human immunodeficiency virus GP120.

11. The nucleic acid of claim 1 wherein said second binding domain is a region of the N u oncogene responsible for homodimer formation.

12. The nucleic acid of claim 1 wherein said second binding domain is a subunit of an adhesion glycoprotein or a functional portion thereof.

13. A substantially pure protein encoded by said nucleic acid of claim 1.

14. A recombinant vector comprising said nucleic acid sequence of claim 1.

15. A recombinant human cytomegalovirus mutant comprising a genome lacking a first nucleic acid sequence encoding a functional first binding domain for class I MHC and having a second nucleic acid sequence encoding a second binding domain for a target, said first nucleic acid sequence being selected from the group consisting of gene US11, gene US2 and portions thereof.

16. A method for degrading a first protein, comprising:

providing a mammal having cells, said cells having a cytosol;

providing a recombinant nucleic acid encoding a second protein selected from the group consisting of US11 and US2, said second protein lacking a functional binding domain for class I MHC and said second protein having a second binding domain for a target on said first protein; and

administering said recombinant nucleic acid to said mammal under conditions which allow said second protein to interact with said first protein such that said first protein is degraded in said cytosol.

17. A method for treating a disease, comprising:

providing a mammal having a disease;

providing a recombinant nucleic acid encoding a human cytomegalovirus protein selected from the group consisting of

US11 and US2, said protein lacking a functional first binding domain for class I MHC and said protein having a second binding domain for a target; and

administering said recombinant nucleic acid to said mammal in a therapeutically effective amount such that treatment of said disease occurs.

18. The method of claim 17 wherein said disease is selected from the group consisting of a viral infection, a bacterial infection, a malignancy, an autoimmune disease and transplant graft rejection.

19. The method of claim 17 wherein said target is part of a molecule necessary for said disease to persist.

20. The method of claim 17 wherein said target is selected from the group consisting of class II MHC and HIV GP120.

21. The method of claim 17 wherein said nucleic acid is administered in a virus vector or in a non-infectious form.

22. A vaccine composition for treating a disease, comprising:

a therapeutically effective amount of a recombinant nucleic acid encoding a human cytomegalovirus protein selected from the group consisting of US11 and US2, said protein lacking a functional first binding domain for class I MHC and said protein having a second binding domain for a target; and

a pharmaceutically acceptable carrier.

23. The vaccine composition of claim 22, further comprising an adjuvant.

24. A pharmaceutical composition for treating a disease, comprising:

a therapeutically effective amount of a recombinant nucleic acid encoding a human cytomegalovirus protein selected from the group consisting of US11 and US2, said protein lacking a

functional first binding domain for class I MHC and said protein having a second binding domain for a target; and

a pharmaceutically acceptable carrier.

25. A method for degrading class I MHC so as to treat an autoimmune disease, comprising:

providing a mammal having an autoimmune disease, said mammal having cells capable of synthesizing class I MHC;

providing a recombinant nucleic acid encoding a human cytomegalovirus protein selected from the group consisting of US11, US2, and functional portions thereof; and

administering a therapeutically effective amount of said recombinant nucleic acid to said mammal so as to degrade class I MHC such that treatment of said autoimmune disease occurs.

26. A vaccine composition for treating an autoimmune disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding a human cytomegalovirus protein selected from the group consisting of US11 protein, US2 protein, and functional portions thereof, and a pharmaceutically acceptable carrier.

27. The vaccine composition of claim 26, further comprising an adjuvant.

28. A pharmaceutical composition for treating an autoimmune disease comprising a therapeutically effective amount of a recombinant nucleic acid encoding a human cytomegalovirus protein selected from the group consisting of US11 protein, US2 protein, and functional portions thereof, and a pharmaceutically acceptable carrier.

29. A method for degrading class I MHC so as to treat tissue graft rejection, comprising:

providing a mammal having a tissue graft;

providing a recombinant nucleic acid encoding a human cytomegalovirus protein selected from the group consisting of US11, US2, and functional portions thereof; and

administering a therapeutically effective amount of said

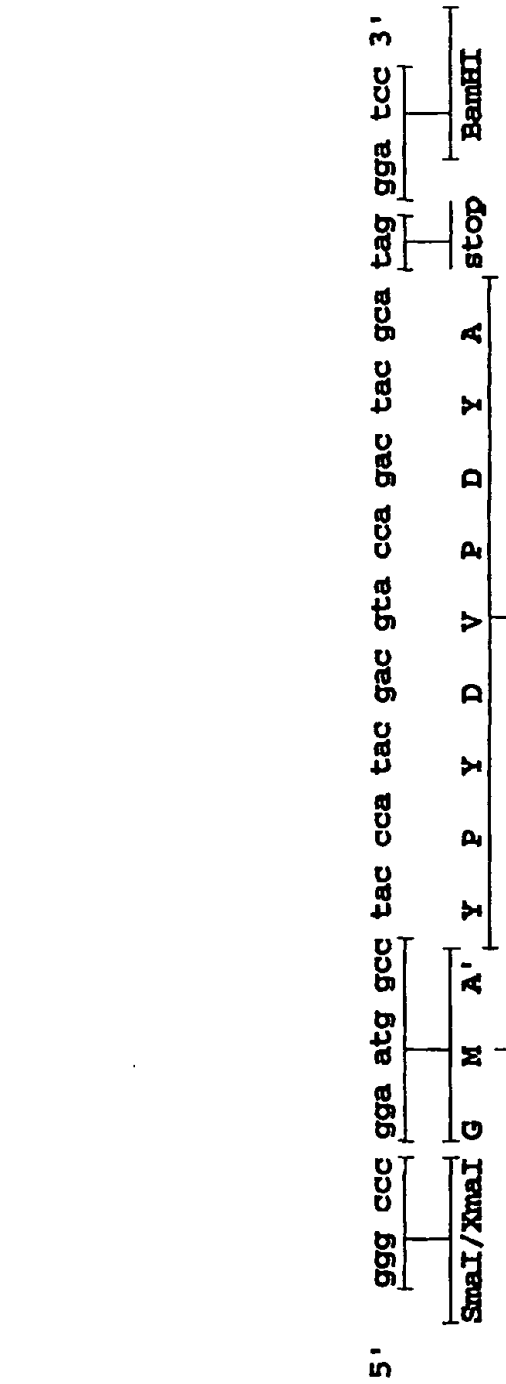
recombinant nucleic acid to said mammal so as to degrade class I MHC such that treatment of said tissue graft occurs.

30. A method for degrading a first protein, comprising:

providing a mammal having cells, said cells having a cytosol;

providing a recombinant nucleic acid encoding a second protein selected from the group consisting of US11, US2, and functional portions thereof, said second protein having a binding domain that is capable of interacting with said first protein; and

administering said recombinant nucleic acid to said mammal under conditions which allow said second protein to interact with said first protein such that said first protein is degraded in said cytosol.



SEQ. ID NO:11

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03606

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 47/30; C07K 14/045; C12N 15/38, 15/62, 15/85, 15/86, 15/87

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.7, 235.1, 320.1; 514/44; 530/350; 536/23.4, 23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

search terms: cytomegalovirus, HCMV, CMV, US11, US2, dedirect, cytosol, degrade, MHC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 96/04384 A1 (AMERICAN CYANAMID COMPANY) 15 February 1996, page 13, lines 19-30; page 14, lines 20-32; page 15, lines 16-24; and claims 1 and 8 on pages 35 and 36.	15,19,30 ---------- 25-28
X --- Y	WO 96/04383 A1 (AMERICAN CYANAMID COMPANY) 15 February 1996, page 3, lines 13p-18; page 14, lines 7-15; page 21, lines 15-31 and claims 4 and 38.	15, 29, 30 ---------- 25-28
Y	YORK et al. A Cytosolic Herpes Simplex Virus Protein Inhibits Antigen Presentation to CD8+ T Lymphocytes. Cell. 20 May 1994, Vol. 77, pages 525-535, especially page 533.	25-30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 JUNE 1997	07 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KEITH C. FURMAN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03606

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	JONES et al. Human Cytomegalovirus US3 Impairs Transport and Maturation of Major Histocompatibility Complex Class I Heavy Chains. Proc. Natl. Acad. Sci. USA. October 1996, Vol. 93, pages 11327-11333. See the whole publication, especially the abstract.	1-30
A, P	MACHOLD et al. The HCMV Gene Products US11 and US2 Differ in Their Ability to Attack Allelic Forms of Murine Major Histocompatibility Complex (MHC) Class I Heavy Chains. J. Exp. Med. 20 January 1997, Vol. 185, No. 2, pages 363-366. See whole publication, especially the abstract.	1-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03606

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/199.1; 435/69.7, 235.1, 320.1; 514/44; 530/350; 536/23.4, 23.72